

# An Investigation of the Metabolism of Valine to Isobutyl Alcohol in *Saccharomyces cerevisiae*\*

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The metabolism of valine to isobutyl alcohol in yeast was examined by  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy and combined gas chromatography-mass spectrometry. The product of valine transamination,  $\alpha$ -ketoisovalerate, had four potential routes to isobutyl alcohol. The first, via branched-chain  $\alpha$ -ketoacid dehydrogenase to isobutyryl-CoA is not required for the synthesis of isobutyl alcohol because abolition of branched-chain  $\alpha$ -ketoacid dehydrogenase activity in an *lpd1* disruption mutant did not prevent the formation of isobutyl alcohol. The second route, via pyruvate decarboxylase, is the one that is used because elimination of pyruvate decarboxylase activity in a *pdcl pdc5 pdc6* triple mutant virtually abolished isobutyl alcohol production. A third potential route involved  $\alpha$ -ketoisovalerate reductase, but this had no role in the formation of isobutyl alcohol from  $\alpha$ -hydroxyisovalerate because cell homogenates could not convert  $\alpha$ -hydroxyisovalerate to isobutyl alcohol. The final possibility, use of the pyruvate decarboxylase-like enzyme encoded by *YDL080c*, seemed to be irrelevant, because a strain with a disruption in this gene produced wild-type levels of isobutyl alcohol. Thus there are major differences in the catabolism of leucine and valine to their respective "fusel" alcohols. Whereas in the catabolism of leucine to isoamyl alcohol the major route is via the decarboxylase encoded by *YDL080c*, any single isozyme of pyruvate decarboxylase is sufficient for the formation of isobutyl alcohol from valine. Finally, analysis of the  $^{13}\text{C}$ -labeled products revealed that the pathways of valine catabolism and leucine biosynthesis share a common pool of  $\alpha$ -ketoisovalerate.

We recently reported on the catabolism of leucine by *Saccharomyces cerevisiae*, showing that this proceeds by transamination to  $\alpha$ -ketoisocaproate and then to isoamyl alcohol using a pyruvate decarboxylase-like enzyme encoded by *YDL080c* (1). This route in yeast differs from that used in other eukaryotes for catabolism of the branched-chain amino acids leucine, isoleucine, and valine and that has been well understood for many years (2). In most eukaryotes the first step is a transamination in which  $\alpha$ -ketoglutarate accepts the amino group (from leucine, isoleucine, and valine) producing glutamate and  $\alpha$ -ketoisocaproic acid,  $\alpha$ -keto- $\beta$ -methylvaleric acid, and  $\alpha$ -ketoisova-

leric acid (respectively). The next step involves oxidative decarboxylation of the keto acids by branched-chain  $\alpha$ -ketoacid dehydrogenase to the corresponding acyl-CoA derivatives. Further steps yield, ultimately, metabolites, all of which can enter the tricarboxylic acid cycle (2).

In yeasts branched-chain amino acids can serve as sole source of nitrogen but not carbon (3, 4). The predominant view, until our recent work on leucine catabolism (1), has been that yeasts first use transamination, but that decarboxylation of the keto acids proceeds via a "carboxylase" to an aldehyde that is then reduced in an NADH-linked reaction producing the appropriate "fusel" alcohol (3–5). This explanation, sometimes called the "Ehrlich pathway" to honor the originator of the ideas (6), which were slightly modified later (7), has numerous problems. The Ehrlich pathway has never been properly proven as the route by which the branched-chain amino acids are catabolized in yeasts. Crucially, the wrongly named carboxylase (which should at least be called a "decarboxylase") has never been identified. A few authors have assumed that pyruvate decarboxylase is responsible (5), without proof. Some metabolic schemes even envisage a blending of catabolic and biosynthetic pathways (8). These and other considerations have led us to undertake a thorough re-examination of the catabolism of the branched-chain amino acids in *S. cerevisiae* which has confirmed that the first step is transamination (9). Two distinct aminotransferases have been shown to function both in amino acid biosynthesis and catabolism. One is mitochondrial (*TWT1* gene product) and one is cytosolic (*TWT2* gene product) (10, 11). The genetic nomenclature here is somewhat confusing: *TWT1* (open reading frame *YHR208w*) and *TWT2* (*YJR148w*) have been referred to as *BAT1* and *BAT2* for branched-chain amino acid transaminase, respectively, (11) although the acronym "BAT" had already been coined. The two genes have also been referred to as *ECA39* and *ECA40*, respectively, because they are homologous to similarly named genes in several other eukaryotic systems (10). The mitochondrial isozyme is highly expressed during logarithmic phase and is repressed during stationary phase whereas the cytosolic isozyme has the opposite pattern of expression (11). Surprisingly, *twt1 $\Delta$  twt2 $\Delta$*  double mutants still possess high levels of branched-chain amino acid aminotransferase activity in the cytosol (11) indicating that other enzyme(s) exist with this activity. At present it is not clear what these enzymes are. Branched-chain  $\alpha$ -ketoacid dehydrogenase has also been demonstrated in *S. cerevisiae* (12). It has been purified from this yeast, and a number of its properties have been characterized (13).

Our analysis of the metabolism of leucine to isoamyl alcohol using  $^{13}\text{C}$  NMR spectroscopy showed that pyruvate decarboxylase was not required for this conversion because the complete elimination of pyruvate decarboxylase activity in a *pdcl pdc5 pdc6* triple mutant did not reduce the levels of isoamyl alcohol produced. Instead, a pyruvate decarboxylase-like enzyme en-

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TABLE I  
Yeast strains used

Strain	Complete genotype	Source or Ref.
IWD72	MAT $\alpha$	(12)
JRD719	MAT $\alpha$ <i>ura3 trp1</i>	J. R. Dickinson
YSH56-1-4A	MAT $\alpha$ <i>leu2 ura3 trp1 pdc1<math>\Delta</math>::LEU2 GAL</i>	S. Hohmann <sup>a</sup>
YSH5.127.-17C	MAT $\alpha$ <i>leu2 ura3 trp1 pdc1<math>\Delta</math>::LEU2 pdc5<math>\Delta</math>::URA3 pdc6<math>\Delta</math>::TRP1 GAL</i>	S. Hohmann <sup>a</sup>
FY1679-YDL080c(a)	MAT $\alpha$ <i>ura3 ydl080c::Kan<sup>r</sup></i>	(1)
JRD815-6.1	MAT $\alpha$ <i>leu2 ura3 trp1 pdc1<math>\Delta</math>::LEU2 pdc5<math>\Delta</math>::URA3 pdc6<math>\Delta</math>::TRP1 ydl080c::Kan<sup>r</sup></i>	(1)
52.1.1	MAT $\alpha$ <i>leu2 ura3 trp1 pdc6<math>\Delta</math>::TRP1</i>	This work
52.3.3	MAT $\alpha$ <i>leu2 trp1 pdc5<math>\Delta</math>::URA3</i>	This work
MML22	MAT $\alpha$ <i>leu2 ura3 his3 ade2 lys2 met8 gal80::HIS3 lpd1::URA3</i>	(18)
53.2.1	MAT $\alpha$ <i>ura3 ade2 lpd1::URA3</i>	This work

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coded by *YDL080c* appears to be the major route from  $\alpha$ -ketoisocaproate to isoamyl alcohol (1). Quite recently ter Schure *et al.* (14) confirmed part of our conclusions by showing that pyruvate decarboxylase was capable of decarboxylating  $\alpha$ -ketoisocaproic acid,  $\alpha$ -keto- $\beta$ -methylvaleric acid, and  $\alpha$ -ketoisovaleric acid *in vitro*, but that a *pdc1 pdc5 pdc6* homozygous diploid still produced the fusel alcohols when grown in a medium containing a mixture of all three of the branched-chain amino acids. It was with this background that we started to determine the metabolic pathways used in the catabolism of valine to isobutyl alcohol.

#### EXPERIMENTAL PROCEDURES

**Strains, Media, and Cultural Conditions**—The strains used are shown in Table I. Strains 52.1.1 and 52.3.3 were constructed by mating YSH5.127.-17C to JRD719 and sporulation of the resultant diploid. Standard genetic techniques were used in the strain construction (15, 16). Because both parental strains in this cross carried *ura3* and *trp1* mutations, haploid progeny segregating either a single *pdc5 $\Delta$ ::URA3* or *pdc6 $\Delta$ ::TRP1* mutation were identified by the ability to grow on glucose minimal medium lacking uracil or tryptophan, respectively. Strain 53.2.1 was constructed by mating MML22 to JRD719. Because both parents in this cross carried *ura3* mutations, haploid progeny segregating the *lpd1::URA3* mutation were recognized by the ability to grow on glucose minimal medium lacking uracil and the inability to grow on any medium in which glycerol was the carbon source (17–19). Enzyme assays (17–19) showed that strain 53.2.1 lacked pyruvate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase, and branched-chain  $\alpha$ -ketoacid dehydrogenase as a consequence of the *lpd1::URA3* mutation. Starter cultures were grown in a medium comprising 1% yeast extract, 2% Bacto-peptone, and 2% carbon source. For studies of valine catabolism cells were grown in minimal medium containing (per liter) 1.67 g of yeast nitrogen base (Difco), 20 g of valine, and either 20 ml of ethanol or 20 g of glucose for the carbon source. Experiments involving <sup>13</sup>C labeling used [2-<sup>13</sup>C]valine (99.9 atom %) from Cambridge Isotope Laboratories (Cambridge, MA). Auxotrophic requirements were supplied as required at 20  $\mu$ g/ml. Liquid cultures were grown in conical flasks filled to 40% nominal capacity in a gyrorotatory shaker. Agar (2%) was used to solidify media. All cell cultures were at 30 °C.

**NMR Analyses**—<sup>13</sup>C NMR spectra were recorded and signals were identified exactly as described previously (1). Chemical shifts ( $\delta$ , ppm) are reported relative to external tetramethylsilane in C<sup>2</sup>HCl<sub>3</sub>; addition of sodium trimethylsilylpropanesulfonate gave a methyl signal at -2.6 ppm under the conditions used here.

**Determination of Isobutyl Alcohol Levels**—Isobutyl alcohol levels were determined in culture filtrates by gas chromatography-mass spectrometry (GC-MS) on a 30-m (0.32-mm internal diameter) fused silica capillary column with a 0.25- $\mu$ m film of Supelcowax 10 (Supelco) in a Voyager GC-MS (Finnigan, Manchester, United Kingdom). The injector temperature was 250 °C, and the samples were chromatographed isothermally at 60 °C; helium was used as the carrier gas at a constant flow rate of 1 ml/min. Standard solutions of isobutyl alcohol gave a linear calibration over the range 0–500  $\mu$ g/ml. Cell extracts were examined for the ability to convert  $\alpha$ -hydroxyisovaleric acid or isobutyric acid into isobutyl alcohol *in vitro* as described previously (1).

#### RESULTS

**Valine Catabolism in a Wild-type Strain**—Fig. 1 shows the <sup>13</sup>C NMR spectrum of a culture supernatant of a wild-type

strain that had been cultured for 23 h in a minimal medium in which glucose was the carbon source and [2-<sup>13</sup>C]valine the sole nitrogen source. The largest signal was C-2 of valine at 60.6 ppm (the <sup>13</sup>C-labeled substrate). A number of resonances were observed due to natural abundance in the valine substrate: C-1 (at 174.3 ppm appearing as a doublet  $J = 53$  Hz due to the adjacent labeled C-2), C-3 (at 29.1 ppm as a doublet  $J = 33$  Hz due to the adjacent labeled C-2), C-4 (18.0 ppm), and C-5 (16.6 ppm). A small signal at 41.6 ppm was due to an unidentified impurity in the [2-<sup>13</sup>C]valine. There were no signals due to residual glucose, while the presence of signals at 57.5 ppm and 16.9 ppm confirmed the production of ethanol. Additional resonances were identified as C-2 of  $\alpha$ -ketoisovalerate (211.4 ppm), C-1 of isobutyrate (186.7 ppm), C-2 of  $\alpha$ -hydroxyisovalerate (77.3 ppm), and C-1 of isobutyl alcohol (69.0 ppm). The small signal due to C-2 of isoamyl alcohol (40.4 ppm) was the result of secondary reactions which are described below but are not relevant to the primary route used in the metabolism of valine to isobutyl alcohol. The resonance at 69.2 ppm (Y in Fig. 1) could not be identified. DEPT analysis showed that it was a carbon atom joined to only one hydrogen. This suggests the carbon atom attached to the OH group in a hydroxy acid or, possibly, a secondary alcohol or diol. The resonances due to C-2 of  $\alpha$ -hydroxyisocaproate, C-2 of lactate, C-2 of malate, C-3 of  $\beta$ -hydroxybutyrate, C-2 of 2-butanol, C-3 of 1,3-butanediol, and also C-3 of  $\alpha$ -isopropylmalate were all very close in standards, but spiking experiments showed that these were not identical with resonance Y in Fig. 1.

The metabolites identified and the positions that were labeled with <sup>13</sup>C suggested the metabolic pathways shown in Fig. 2, all of which involved initial transamination to  $\alpha$ -ketoisovalerate. The first was via branched-chain  $\alpha$ -ketoacid dehydrogenase (*route A* in Fig. 2) to yield isobutyryl-CoA, which would then be converted by acyl-CoA hydrolase to isobutyrate and hence isobutyl alcohol. The second possibility was via pyruvate decarboxylase (*route B*). The occurrence of  $\alpha$ -hydroxyisovalerate could be explained by the existence of *route C* involving  $\alpha$ -ketoisovalerate reductase (data not shown) analogous to the conversion of  $\alpha$ -ketoisocaproate to  $\alpha$ -hydroxyisocaproate seen previously (1). *Route D* envisaged a pyruvate decarboxylase-like enzyme such as that encoded by *YDL080c* that is involved in the formation of isoamyl alcohol from  $\alpha$ -ketoisocaproate (1). Just as in the previous study (1) we could not conceive a metabolic route between  $\alpha$ -ketoisocaproate and isovalerate using known or potential enzymes, neither could we for the analogous conversion of  $\alpha$ -ketoisovalerate to isobutyrate (theoretical *route E* in Fig. 2). Thus, this last possibility was discarded and the subsequent experiments were devised in order to distinguish between the four remaining possibilities.

**Elucidation of the Pathway from  $\alpha$ -Ketoisovaleric Acid to Isobutyl Alcohol**—The wild-type strain IWD72, mutant strain YSH5.127.-17C, which completely lacks pyruvate decarboxyl-

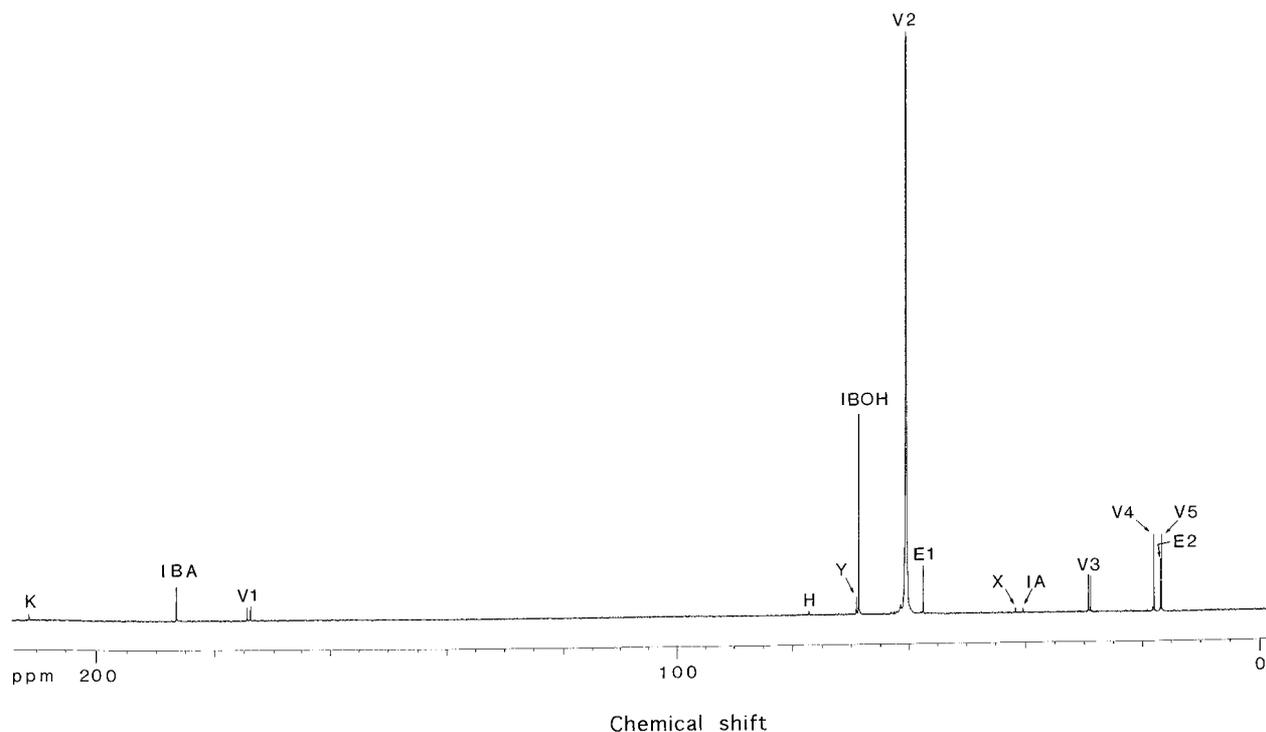


FIG. 1. The  $^{13}\text{C}$  NMR spectrum of a culture supernatant of the wild type strain IWD72. Cells were cultured in ethanol minimal medium with valine as sole nitrogen source. The resonances marked are: V1-V5, C-1 to C-5, respectively, of valine; K, C-2 of  $\alpha$ -ketoisovalerate; IBA, C-1 of isobutyrate; H, C-2 of  $\alpha$ -hydroxyisovalerate; IBOH, C-1 of isobutyl alcohol; E1, E2, C-1 and C-2 of ethanol, respectively; IA, C-2 of isoamyl alcohol; X, impurity present in valine; Y, unidentified resonance.

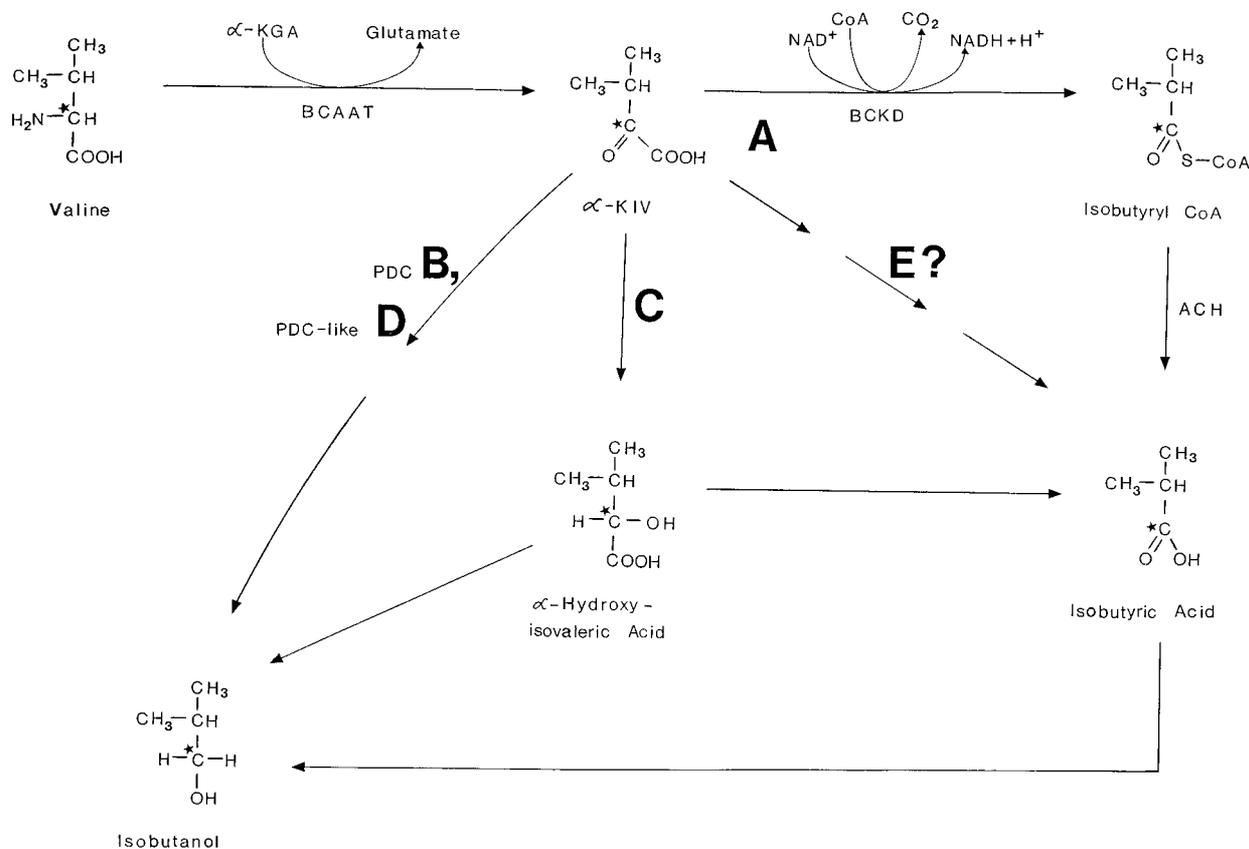


FIG. 2. Potential metabolic routes for the metabolism of valine to isobutyl alcohol. The asterisks indicate carbon atoms in intermediates that were labeled with  $^{13}\text{C}$  in the wild-type strain. Enzyme activities which are already known are abbreviated. BCAAT, branched-chain amino acid amino transferase; BCKD, branched-chain  $\alpha$ -ketoacid dehydrogenase; ACH, acyl-CoA hydrolase; PDC, pyruvate decarboxylase.

FIG. 3. Growth of various strains in minimal medium with valine as sole nitrogen source. ●, wild-type strain IWD72; ■, mutant strain YSH5.127.-17C; ▲, mutant strain FY1679-YDL080c(a); □, mutant strain JRD815-6.1; △, mutant strain 53.2.1.

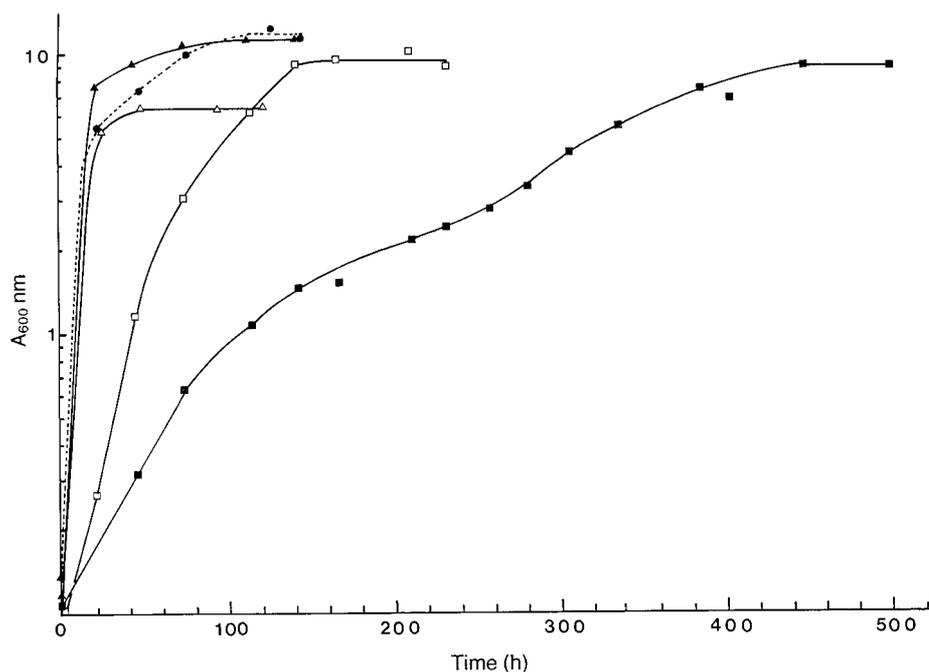


TABLE II

Amount of isobutyl alcohol produced by various strains growing in minimal medium with valine as sole source of nitrogen

Isobutyl alcohol content was determined in the deceleration phase when it was maximal (see Fig. 4). Values are the means of duplicate determinations. Differences between samples were <5%.

Strain	Relevant genotype	Carbon source	Isobutyl alcohol	
			$\mu\text{g/ml}$	$\mu\text{g}/10^6 \text{ cells}$
IWD72	Wild-type	Glucose	3020	41
FY1679-YDL080c(a)	<i>yd1080c::Kan<sup>r</sup></i>	Glucose	3080	40.2
YSH5.127.-17C	<i>pdc1 pdc5 pdc6</i>	Ethanol	18	0.3
JRD815-6.1	<i>pdc1 pdc5 pdc6 yd1080c::Kan<sup>r</sup></i>	Ethanol	57	0.6
YSH56-1-4A	<i>pdc1</i>	Glucose	378	4
52.1.1	<i>pdc6</i>	Ethanol	274	3.1
52.3.3	<i>pdc5</i>	Ethanol	300	6.4
53.2.1	<i>lpd1</i>	Glucose	400	7.7

ase activity due to disruptions in all three genes for pyruvate decarboxylase (*PDC1*, *PDC5*, *PDC6*) (20), FY1679-YDL080c(a), which is disrupted in open reading frame *YDL080c* (which we have called *KID1* (1) and is also known as *THI3* (21)), JRD815-6.1, which is disrupted in *PDC1*, *PDC5*, *PDC6*, and *YDL080c* (1), and 53.2.1, which lacks lipoamide dehydrogenase due to a disruption in the *LPD1* gene (18), were all capable of growth in minimal medium with valine as sole nitrogen source (Fig. 3). The strain lacking all three isoenzymes of pyruvate decarboxylase produced very little isobutyl alcohol (Table II). Strain JRD815-6.1, which lacked the *YDL080c*-encoded decarboxylase in addition to lacking all three isoenzymes of pyruvate decarboxylase, produced a similarly low level of isobutyl alcohol to strain YSH5.127.-17C, which lacked all three pyruvate decarboxylases; whereas the strain that lacked only the *YDL080c*-encoded decarboxylase produced wild-type levels of isobutyl alcohol (Table II). This leads immediately to the conclusion that the catabolism of valine to isobutyl alcohol requires pyruvate decarboxylase but not branched-chain  $\alpha$ -ketoacid dehydrogenase nor the *YDL080c*-encoded decarboxylase.

The mutant strain 53.2.1, which lacked lipoamide dehydrogenase and as a consequence of this defect lacks pyruvate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase, glycine decarboxylase, and branched-chain  $\alpha$ -ketoacid dehydrogenase (17–19, 22), produced isobutyl alcohol levels that were not as high as the wild-type or *yd1080c* mutant but were much higher than those of the pyruvate decarboxylase-less mutant. This implies

that a lipoamide dehydrogenase-dependent activity plays some part in setting isobutyl alcohol levels but that lipoamide dehydrogenase is not essential for isobutyl alcohol formation. A direct role of lipoamide dehydrogenase could be as an essential component of branched-chain  $\alpha$ -ketoacid dehydrogenase. However, this can be discounted because neither isobutyric acid nor  $\alpha$ -hydroxyisovaleric acid was converted into isobutyl alcohol in cell-free extracts from wild-type strains that had been grown on valine as sole source of nitrogen (data not shown). Hence, the lack of lipoamide dehydrogenase must cause the lower level of isobutyl alcohol in the *lpd1* mutant by an indirect effect probably due to reduced energy production and consequent reduced growth arising from lack of both pyruvate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase activity. In addition, the absence of pyruvate dehydrogenase means that acetate units do not enter the tricarboxylic acid cycle, resulting in a shortage of  $\alpha$ -ketoglutarate to act as amino group receiver from valine in the initial aminotransferase reaction.

*A Single Pyruvate Decarboxylase Isozyme Is Sufficient for Isobutyl Alcohol Formation from Valine*—Strains YSH56-1-4A (*pdc1*), 52.3.3 (*pdc5*), and 52.1.1 (*pdc6*) all grew well on minimal medium in which valine was the sole source of nitrogen, and all produced similar amounts of isobutyl alcohol (Fig. 4 and Table II). Hence, disruption of any single pyruvate decarboxylase gene resulted in a yeast cell that was able to produce isobutyl alcohol from valine. The *PDC1*-encoded isoenzyme (Pdc1) is the major pyruvate decarboxylase responsible for the

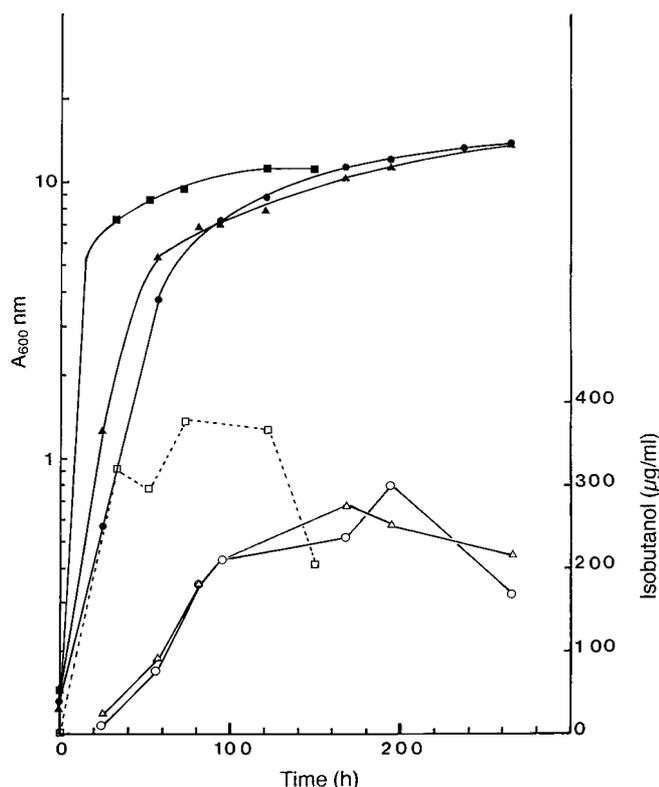


FIG. 4. Growth and isobutyl alcohol formation by strains with single pyruvate decarboxylase mutations in minimal medium with valine as sole nitrogen source. The figure shows  $A_{600 \text{ nm}}$  (solid symbols) and isobutyl alcohol in the medium,  $\mu\text{g/ml}$  (open symbols) in strains YSH56-1-4A (*pdc1*), ■; 52.3.3 (*pdc5*), ●; and 52.1.1 (*pdc6*), ▲. For strain YSH56-1-4A the carbon source was glucose; for strains 52.3.3 and 52.1.1 the carbon source was ethanol.

conversion of pyruvate to acetaldehyde and carbon dioxide. Only *PDC1* is expressed on glucose with autoregulation of *PDC1* and *PDC5*. Thus, when *PDC1* is deleted, *PDC5* is expressed on glucose to about 80% of the level of *PDC1* in a wild type strain. The expression of *PDC1* is 10- to 20-fold lower when glucose levels are low or when ethanol is the carbon source. *PDC6* expression is weak and seems to occur only during growth on ethanol. It is especially required for proper growth initiation of spores germinating on ethanol (20). Using this knowledge one can deduce that the *pdc1* strain grew on glucose using *Pdc5*, the *pdc5* mutant growing on ethanol had an active *Pdc6*, and the *pdc6* mutant growing on ethanol had low pyruvate decarboxylase activity due to *Pdc1*. Thus, in each of these mutants there was only a single pyruvate decarboxylase isozyme operating and in each case the level of pyruvate decarboxylase activity was less than normally present in a wild-type cell. Nevertheless, all of the mutants made moderate amounts of isobutyl alcohol showing that a single pyruvate decarboxylase isozyme is all that is required for isobutyl alcohol formation from valine.

**Secondary Reactions Leading to the Formation of Isoamyl Alcohol**—The occurrence of isoamyl alcohol labeled at C-2 (Fig. 1) is readily explained by the pathway of leucine biosynthesis (Fig. 5).  $\alpha$ -Ketoisovaleric acid labeled at C-2 is converted to  $\alpha$ -isopropylmalate labeled at C-3 by  $\alpha$ -isopropylmalate synthase. This is then converted by  $\beta$ -isopropylmalate dehydratase to  $\beta$ -isopropylmalate labeled at C-3 which is, in turn, converted to  $\alpha$ -ketoisocaproic acid by  $\beta$ -isopropylmalate dehydrogenase. The next step in the leucine biosynthetic pathway is the formation of leucine, which would be labeled at C-3. No signal due to C-3 of leucine was observed, however isoamyl alcohol labeled

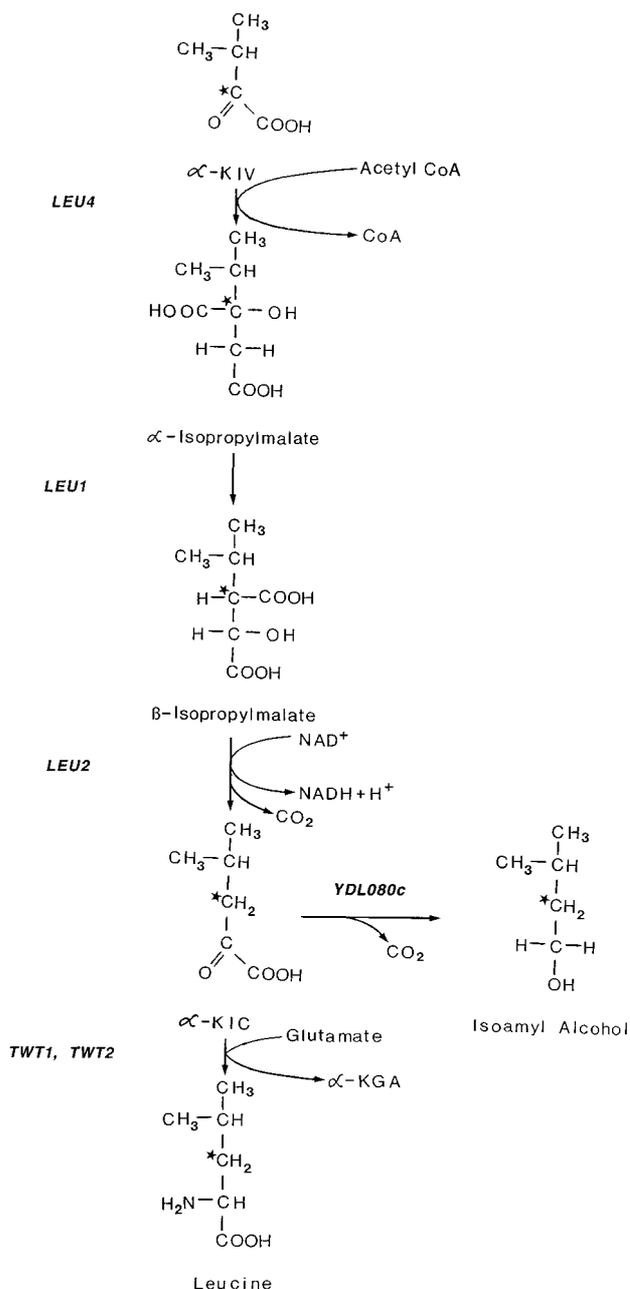


FIG. 5. The pathway of leucine biosynthesis. The asterisks indicate carbon atoms in intermediates that were labeled with <sup>13</sup>C. Enzymes are denoted by the structural genes which encode them. *LEU4*,  $\alpha$ -isopropylmalate synthase; *LEU1*,  $\beta$ -isopropylmalate dehydratase; *LEU2*,  $\beta$ -isopropylmalate dehydrogenase; *TWT1*, *TWT2*, mitochondrial and cytoplasmic isozymes, respectively, of branched-chain amino acid transaminase; *YDL080c* = *KID1* = *THI3*,  $\alpha$ -ketoisocaproate decarboxylase.

at C-2 was present (Fig. 1). This explanation is confirmed by the fact that strain FY1679-YDL080c(a), which lacks the *YDL080c*-encoded  $\alpha$ -ketoisocaproate decarboxylase, makes plenty of isobutyl alcohol but no isoamyl alcohol when valine is the sole nitrogen source (data not shown).

#### DISCUSSION

Analysis of the metabolism of [2-<sup>13</sup>C]valine by the wild type strain suggested four credible routes from  $\alpha$ -ketoisovalerate to isobutyl alcohol (Fig. 2). Experiments using a range of different mutants led to the conclusion that pyruvate decarboxylase is used in the decarboxylation of  $\alpha$ -ketoisovaleric acid. Indeed, any single isozyme of pyruvate decarboxylase is sufficient. This

corresponds to the modern view of the Ehrlich pathway (4–8) and is completely different from the catabolism of leucine in yeast which uses the unique decarboxylase encoded by *YDL080c* to catalyze the decarboxylation of  $\alpha$ -ketoisocaproate (1). It is now clear that neither the catabolism of leucine nor the catabolism of valine to the associated fusel alcohols requires branched-chain  $\alpha$ -ketoacid dehydrogenase. This makes the physiological role of this enzyme in yeast even more mysterious. However, it is known that the activity of branched-chain  $\alpha$ -ketoacid dehydrogenase is greater in complex medium when glycerol is the carbon source than in minimal media containing a branched-chain amino acid (12). The present study involved neither the use of glycerol nor complex media, but was confined to the metabolic routes involved in the catabolism of valine in minimal media. An explanation for all currently known facts is that branched-chain amino acid catabolism utilizes predominantly branched-chain  $\alpha$ -ketoacid dehydrogenase in complex media, but not at all in minimal media.

Growth on valine as sole nitrogen source and the ability to form isobutyl alcohol from it are separate. This was demonstrated by the *pdc1 pdc5 pdc6* triple mutant, which grew on minimal medium with valine as the sole nitrogen source but made no isobutyl alcohol. Hence the valine was used solely as a source of amino group nitrogen to permit the whole of cellular nitrogen metabolism and growth.

The formation of [2-<sup>13</sup>C]isoamyl alcohol from the [2-<sup>13</sup>C] $\alpha$ -ketoisovaleric acid produced by the deamination of valine is explained by utilization of the [2-<sup>13</sup>C] $\alpha$ -ketoisovaleric acid in the leucine biosynthetic pathway. Hence, a mixing of catabolic and biosynthetic pathways accounts for the formation of isobutyl alcohol and isoamyl alcohol, respectively, from valine. Webb

and Ingraham (8) had previously proposed this. The consequences of this for metabolic control within the yeast cell are enormous.

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